Signosis
Innovative Plate Assay Solutions

Human Akt Pathway cDNA Plate Array

Catalog Number AP-0161 **(For Research Use Only)**

Introduction

Akt is a serine/threonine protein kinase that plays important roles in mediating signals for numerous of cell functions including cell growth, differentiation, cell cycle, transcription, and glucose metabolism. The enzyme is activated by PI3K and activated Akt leads to phosphorylation of a wide range of protein substrates such as GSK-3, IKK-β, BAD, MDM2, mTOR, CASP9, p21Cip1, p27Kip1. Differential expression of Akt substrates contributes different phenotypic outcomes. Signosis' Akt pathway-regulated cDNA plate array provides a simple approach to profile the expression of these genes.

Principle of the assay

Signosis' proprietary cDNA plate array is a plate-based hybridization profiling analysis for monitoring the expression of dozens of genes through reverse transcription of mRNA into cDNA. Like array analyses, total RNA is first reverse transcribed into cDNA in the presence of biotin-dUTP in the assay. Targeted genes are then specifically captured onto individual wells on a plate, instead of membranes, through a pre-coated gene-specific oligonucleotide. The captured cDNAs are further detected with streptavidin-HRP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer

The expression level of genes is directly proportional to the luminescent intensity.

Materials provided with the kit

- A 96-well plate coated with 23 different capture oligos (RT)
- Human Akt Primer Mix (-20 °C)
- Reverse transcription buffer mix (-20 °C)
- Reverse transcriptase RT (-20 °C)
- Streptavidin-HRP conjugate (4°C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5xDetection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (4°C)

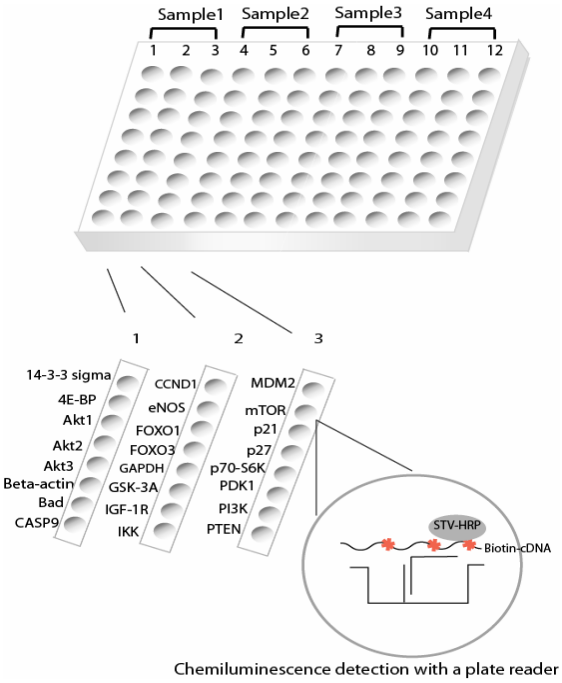

Diagram of human Akt pathway cDNA plate assay

Material required but not provided

PCR machine
Incubator
0.2ml PCR tube
luminometer plate reader
ddH2O (RNAase free)

Reagent preparation before starting experiment

- Dilute 30ml of 5x Plate hybridization wash buffer with 120 ml of dH_2O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Warm up Plate hybridization buffer for two hours to 16 hours at 45 °C until no visible precipitate before use. Stir the solution with 10ml or 5ml pipette to facilitate the dissolving process.
- Dilute 500 times of streptavidin-HRP with blocking buffer before use.

Assay procedure

1. cDNA synthesis using PCR machine
Note: Briefly spin tubes before opening

(1) Sample preparation
X µl 1-10µg total RNA
2 µl Human AKT Reg. Primer Mix
X µl ddH2O

11µl

(2) Incubate for 5 minutes at 65 °C, and chill on ice.

(3) Add 8 µl Reverse transcription buffer mix and 1µl RT to each reaction tube, and incubate for 1 hour at 45 °C.

(4) Heat the reaction to 98 °C for 5 minutes, and chill on ice.

(5) The 20ul cDNA is synthesized and labeled with biotin and ready for hybridization on the plate.

2. Plate hybridization

(1) Remove the sealing film

(2) Arrange the appropriate number of the wells of the plate based on your experiment. The whole plate is divided into 4 repeat sections, Column 1-3, 4-6, 7-9, 10-12 for 4 different samples.

(3) Mix 20ul cDNA with 2.8ml pre-warmed Plate hybridization buffer, and dispense 95ul mixture to each well in a section **immediately**. A reagent reservoir can be used for dispensing cDNA mixture into the wells with a 8 multichannel pipette.

(4) Seal the wells with foil film securely and hybridize at 45 °C for overnight. Ensure the numbers and letters on the plate are clearly visible from under foil seal by pressing the foil down on every single experimental well.

3. Plate detection

(1) Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.

(2) Invert the Hybridization Plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200ul of pre-warmed 1x Plate hybridization wash buffer to each well. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.

(3) Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

(4) Add 200µl of Blocking buffer to each well and incubate for 15 minutes at room temperature with gently shaking.

(5) Invert the plate over an appropriate container to remove block buffer.

(6) Add 20 µl of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for one plate. Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.

(7) Wash the plate 3 times by adding 200ul 1X Detection wash buffer to each well, At each wash, incubate the plate for 10 minutes with gently shaking at room temperature.

(8) Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, invert plate on clean paper towels for 1-2 min to remove excessive liquid.

(9) Freshly prepare the substrate solution:
For the whole plate:
1ml Substrate A
1ml Substrate B
8ml Substrate dilution buffer

(10) Add 95µl substrate solution to each well and incubate for 1 min.

(11) Place the plate in the luminometer. Allow plate to sit inside machine for 5 min before reading. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 minutes.

Example of Analysis Data

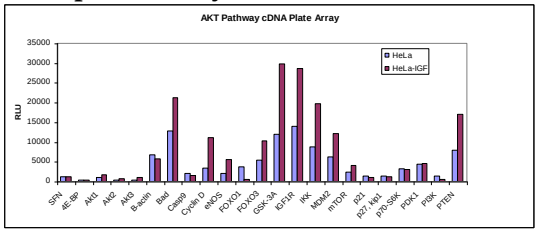

Figure1: Akt Pathway cDNA plate assay for analysis of 23 genes.

HeLa cells were treated with and without 100ng/ul IGF for 4 hours respectively. Total RNAs were prepared. RNAs were subjected to AKT Pathway cDNA plate assay, and detected with chemiluminence by a plate reader.

Diagram of human Akt cDNA plate assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	14-3-3 sigma	CCND1	MDM2	14-3-3 sigma	CCND1	MDM2	14-3-3 sigma	CCND1	MDM2	14-3-3 sigma	CCND1	MDM2
B	4E-BP	eNOS	mTOR	4E-BP	eNOS	mTOR	4E-BP	eNOS	mTOR	4E-BP	eNOS	mTOR
C	Akt1	FOXO1	p21	Akt1	FOXO1	p21	Akt1	FOXO1	p21	Akt1	FOXO1	p21
D	Akt2	FOXO3	p27, kip1	Akt2	FOXO3	p27, kip1	Akt2	FOXO3	p27, kip1	Akt2	FOXO3	p27, kip1
E	Akt3	GAPDH	p70-S6K	Akt3	GAPDH	p70-S6K	Akt3	GAPDH	p70-S6K	Akt3	GAPDH	p70-S6K
F	B-actin	GSK-3A	PDK1	B-actin	GSK-3A	PDK1	B-actin	GSK-3A	PDK1	B-actin	GSK-3A	PDK1
G	Bad	IGF1R	PI3K	Bad	IGF1R	PI3K	Bad	IGF1R	PI3K	Bad	IGF1R	PI3K
H	CASP9	IKK	PTEN	CASP9	IKK	PTEN	CASP9	IKK	PTEN	CASP9	IKK	PTEN